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Date: June 9, 2006 To: PTO	From: Csaba Henter/Anthony J. Zelano MILLEN, WHITE, ZELANO & BRANIGAN, P.C. Arlington Courthouse Plaza I 2200 Clarendon Blvd., Suite 1400 Arlington, VA 22201 (U.S.A.) (Fax: 703-243-6410)
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Re: U.S. Patent Application No. 10/694,847 Your Ref: 44 534 K (Parent Reference) Our Ref: KOENIG-0002-D01	
Total No. of Pages: 2; if you do not receive all pages, please call (703) 243-6333	

Dear Examiner Rooke:

Further to our conversation, please amend claims 23 and 29 by removing the term "or prevented" from each of these claims.

The term written out for PTCA/PTA from claim 24 and elsewhere in the application is Percutaneous Transluminal Coronary Angioplasty / Percutaneous Transluminal Angioplasty. Please make changes where necessary.

If you have any questions, please do not hesitate to contact us.

Very truly yours,

Csaba Henter

K:\koenig\2D1\fax to Examiner.dot

Amendments to the Specification

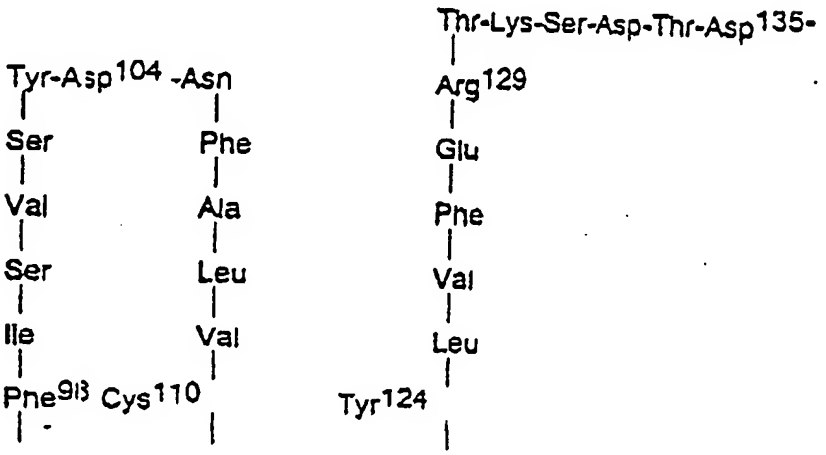
Please insert the paragraph below the title and prior to the first paragraph of the specification.

This application is a divisional of U.S. Application No. 09/445,214 filed 5/3/00, now abandoned, which is a 371 of PCT/EP98/03356 filed on 6/5/98.

Amendments to the Specification:

Please replace the second paragraph on page 11 with the following amended paragraph:

The partial sequence of triabin, which binds to thrombin, read:



SEQ ID NO:1 SEQ ID NO:2

Please replace the first paragraph on page 12 with the amended paragraph below.

By specific exchange of some amino acids and linkage of the sequences, a peptide was set forth in which the amino acids are spaced at intervals that are similar to the partial sequences of triabin that bind to thrombin. In particular, the phenylalanine in triabin at 98-position was exchanged for lysine, which now represents the first amino acid in the peptide. Cysteine 110 in the triabin was replaced by asparaginic acid in the peptide. The carboxyl side group of the asparaginic acid is ~~fixed~~ linked to the amino side group of lysine (1-position in the peptide), so that a cyclic connection is produced. The asparaginic acid is connected to the second partial sequence of triabin (124-135), which binds to thrombin. The peptide has the following sequences:

Agnes Woolwine 06/01/2006

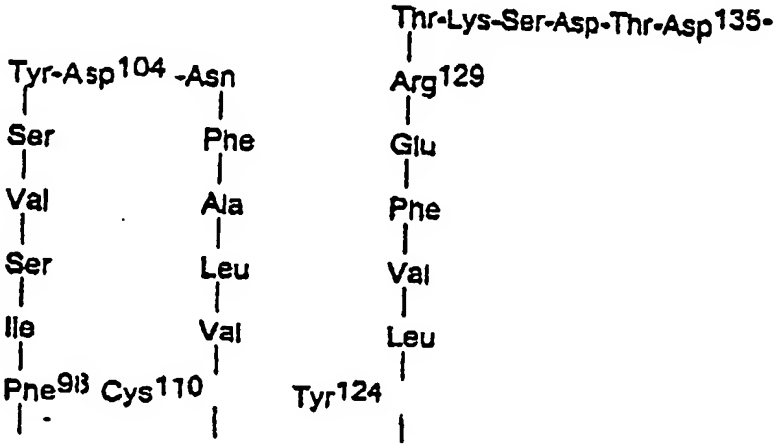
Agnes Poole 06/09/2006

Example 1

1. ~~D t rmination~~ Determination of ~~th~~ the Crystal Structure of a Complex that Consists of Thrombin and Triabin

Purified triabin and thrombin were added together in 20 mmol of sodium acetate, 25 mmol of sodium chloride, pH 5.5. Crystals of the complex of triabin and thrombin formed in a hanging drop, which contained 50 mmol of sodium acetate, pH 4.7, 100 mmol of ammonium sulfate, 0.01% sodium nitrite and 8% PEG 4000. The structure of the crystals was determined by means of x-ray analysis. The amino acids, which form interactions with thrombin, were determined from these structural data. It has been shown that these amino acids are found in areas that form a ß-folded-sheet structure.

The partial sequences of triabin, which bind to thrombin, read:



area of amino acids 98-103 (named chain 1), amino acids 105-110 (chain 2) and amino acids 124-135 (chain 3) must be sterically stabilized. This could be achieved by different modifications of the original areas according to the following batches.

2. Stabilization of the Peptides

A. Stabilization of Chains 1 and 2

The stabilization was carried out either by

1. Exchange of the amino acids phenylalanine in triabin in 98-position and/or cysteine in 110-position for amino acids, which allow a linkage via the side chain (e.g., Lys-Asp, Cys-Cys, ornithine-Glu)

or by

2. Exchange of Cys110 for Asp and linkage of Phe98 and this Asp by a "ß-turn mimetic agent." The structures of ß-turn mimetic agents and their use are described in detail in U. Egner et al. (1997) Pesticide Science, in Press.

Agnes Padie 06/05/06

→**B. ~~The~~ Stabilization of Chains 2 and 3**

The stabilization was carried out either by

1. Exchange of the L-Tyr in 124-position for a D-Tyr (configuration isomer) and linkage of amino acids in 110-position (in the triabin Cys) with that in 124-position (in the triabin Tyr) by a peptide bond

or by

2. Exchange of the L-Tyr in 124-position for a D-Tyr and linkage of the amino acid in 110-position (in the triabin Cys)

Agnes Roelle 06/09/06 ⇒

1. Synthesis of ~~th~~ ~~Peptid~~ the Peptide

The synthesis was carried out according to standard methods in a peptide-synthesis machine of Applied Biosystems.

Sequence: Lys-Ile-Ser-Val-Ser-Tyr-Asp-Asn-Phe-Ala-Leu-Val-Asp-D-Tyr-Leu-Val-Phe-Glu-Arg-Thr-Lys-Ser-Asp-Thr-Asp

2. Cleavage of the Dde and O-All Groups

In addition, the resin was washed twice with DMSO/DCM (1:1) and allowed to steep in this solution for 30 minutes. Then, palladium (0.1 mol/mol of peptide) and DPPF (0.1 mol/mol of peptide) and acetic acid (10-fold excess) were added. $Sn(Bu)_3H$ was added in 5 portions (a total of 5-fold excess) within 10 minutes. The mixture was stirred for 20 minutes, then suctioned off and washed with DMSO/DCM and DCM.

3. Cyclization

The resin was pre-steeped in DMF for 30 minutes, then DIPEA (8-fold excess), TBTU (2-fold excess) and HOBT (2-fold) were added and stirred overnight. The resin was suctioned off and washed with DMF and ether.

4. Cleavage of Resin and Cleavage of the Residual Protective Groups

Phenol, ethyldithiol, thioanisole, H_2O and TFA were added to the resin, and the reaction mixture was stirred for 4 hours at 37°C. The peptide was precipitated with t-butylether, centrifuged off and dried under nitrogen.

Apps Rodw 06/03/2006

Sample Application 3

Measurement of ~~th~~ the ~~Fibrinog n Cl avag~~ Fibrinogen cleavage

Microtiter plates were coated first with bovine serum albumin. Then, 100 µl of triabin solution (0.1-10 µmol/l 10 mmol of Na H_2PO_4, pH 7.4) was added to the microtiter plate, and 100 µl of reaction buffer (20 mmol of HEPES, 0.15 M of NaCl, pH 7.4), 20 µl of $CaCl_2$ solution (20 mmol of $CaCl_2$ in H_2O) and 20 µl of thrombin solution (0.012 IU) were pipetted into it. After an incubation at 37°C for 2 minutes, 100 µl of fibrinogen (10 mg in 2 ml of reaction buffer) was added and incubated for 40 minutes at 37°C. Then, the extinction was measured at 405 nm. As a control value (100% thrombin activity), 100 µl of reaction buffer was used in a batch instead of the triabin solution. The measured values with triabin were related to these values and expressed as % of inhibition.